

Response Of Geraniol Derivative And Cymbopogon Citratus Leaves Extract Against Periimplantitis Causing Pathogens

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Abstract

Periimplantitis due to pathogenic bacteria is the chief cause for the dental implants failure. Evidences over *S. aureus* and *E. coli* to trigger periimplantitis, antimicrobial potential of Cymbopogon citratus and geraniol intended present study to compare the antimicrobial potential of hydrazide derivative of geraniol (HDG) and Cymbopogon citratus leaves extract (CCLE) against periimplantitis causing pathogens (PCP). Present study involved synthesis of HDG and preparation of CCLE. The HDG was characterized using ATR-IR, ¹H-NMR and Mass spectrometric data. Both HDG and CCLE were tested for antimicrobial potential against periimplantitis causing pathogens (PCP), namely: *Escherichia coli* and *Staphylococcus aureus*. Among both, the HDG exhibited much higher antimicrobial potential when compared with CCLE. Based on the resultant data present study concludes that HDG possess high inhibition potential against PCP and recommends that HDG should be further evaluated to support its clinical importance.

Keywords: Periimplantitis, geraniol, Comparison, extract, and antimicrobial

INTRODUCTION

Bacterial contamination of dental implants (DI) in oral cavity is a common problem. Facts suggests, that annually around 10 million new DI are placed globally. Peri-implantitis (PI) is a major threat for the DI success [1]. Human microbiota comprises 1:1 bacteria and human cells, little disturbance in such ratio may activate bacterial periimplantitis causing pathogens (PCP) [2-5]. Evidence suggests role of *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) in PI. Long-term administration of conventional antibiotics against various infections lead to multiple drug resistance and high mortality risk [6-9]. To combat such problem, one can use synthetic or phytoproducts. Terpenes are known to possess high antibacterial activity [10]. Literary evidences support plants products as an effective antibacterial agent [11-15], thereby can be used to combat PI. Plants are

known to possess numerous biological activities, so can be used in wide range of infections, disorders and diseases [16-39]. Several research highlighted numerous biological activities of plants [40-104]. A large number of studies revealed increase in biological potential of plant products together when combined with nanotechnology [105-129]. Literary facts suggest several synthetic moieties to possess high antimicrobial potential [130-142]. Numerous patents on plants and their products have been granted attributed to their higher biological activities [143-182]. Therefore, present study was intended to determine the antimicrobial potential of *Cymbopogon citratus* leaves extract (CCLE) and geraniol derivative against PCP.

MATERIAL AND METHODS

Materials

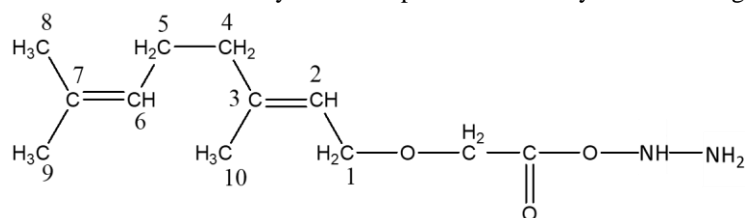
The melting point of synthesized compound was determined using Thomas Hoover apparatus. IR spectra was recorded ATR-IR, Perkin Elmer, ¹H-NMR on Bruker, DPX 300 and mass spectra on MASPEC (MSW/9629). Purity of synthesized compound was checked by TLC aluminium sheets – silica gel 60 F254 (0.2 mm). Plant material was collected from the local market of Sungai Petani, Malaysia. Chemicals, and solvents were procured from the SD Fine, Sigma-Aldrich, and Merck Ltd.

Preparation of Plant Extract

The CCLE was prepared as per the standard procedure in the literature [8]. Briefly, *Cymbopogon citratus* leaves free of decay were collected from the province of Sungai Petani, Kedah state, Malaysia and washed with fast flowing tap water, followed by air drying, mincing into small pieces; and macerated for 15 days using hydroalcoholic solvent (50:50). The mixture was filtered using double muslin cloth and a filter paper (Whatman No. 1) and the filtrate was dried to offer dark brown coloured CCLE. The obtained CCLE was stored at 4°C in refrigerator for further evaluation of its antimicrobial activity against PCP.

Procedure for the synthesis of hydrazone derivative of geraniol (HDG)

The synthesis of HDG was done as per the standard protocol with slight modifications [130-140]. Briefly, the ester derivative of geraniol (that was synthesized by refluxing geraniol with ethylchloroacetate), was refluxed with equimolar concentration of hydrazine hydrate for 4 hours and cooled overnight to offer needle like crystals of HDG. The obtained crystals were purified and recrystallized using methanol.



Response of CCLE and HDG against PCP

Preparation of bacterial culture

Bacterial strains of *Staphylococcus aureus* and *Escherichia coli* were used for the antimicrobial experiment. The prepared stock culture of microorganism was maintained at 4°C. Subcultures were prepared by transferring loopful of microorganisms' colonies from stock cultures into the nutrient broth and incubated for 24 hours at 37°C in the incubator. The broth turbidity indicated the microbial growth [11,12].

Well Diffusion Method

The inhibitory potential of the prepared CCLE and HDG against PCP was determined using well diffusion method-based zone of inhibition. The experimental protocol was followed as per the standard references with slight modifications [132-134]. Briefly, 20 µl of nutrient broth containing broth organism was poured into Muller Hinton agar plate, that was spread uniformly using L-shape rod. The wells were made on the agar medium with cork borer of 5 mm in diameter which was previously sterilized using autoclave at 121°C for one hour. Each 50 µl of CCLE and HDG were pipetted separately into the cup made on the agar plate. In the agar plate a few wells

for CCLE, HDG, standard and control. These plates contained the antibiotic streptomycin (standard) and tween 80 (control) solution for the purpose of comparison with the CCLE and HDG. All the plates were incubated for 24 hours at 37°C. The diameter of zone of inhibition around wells was measured in millimetres (mm) in triplicate and average values were calculated.

Preliminary Phytochemical screening of CCLE

The CCLE was subjected to preliminary phytochemical screening for the detection of various plant constituents. The prepared extract was screened for the presence of alkaloids, carbohydrates, flavonoids, glycosides, proteins, tannins, and phenols as per the procedure given in standard references [58,59].

RESULTS

Synthesis of HDG

White needle shaped crystals; Yield 79%; mp 38-39 °C; ATR-IR: 3250, 3010, 2970, 2924, and 1740 cm⁻¹; ¹H-NMR δ (ppm): 2.01 (4H, m, CH₂-4,5), 1.73 (9H, s, CH₃-8,9,10), 4.08 (2H, d, CH₂), 4.14 (2H, d, O-CH₂), 4.35 (2H, s, O-CH₂-CO), 5.22 (1H, t, CH-6), 5.26 (1H, t, CH-2), 4.33 (2H, br, NH₂), 9.50 (1H, br, NH); MS: m/z: 242 (M⁺).

Response of CCLE and HDG against PCP

In present study, the prepared CCLE and evaluated for their inhibitory potential against PCP such as *S. aureus* and *E. coli* using agar well diffusion for measurement of zone of inhibition. The prepared CCLE and HDG were evaluated for their antimicrobial potential against bacterial strains of *S. aureus* and *E. coli*. The results so obtained are given in table 1.

Table 1: Zone of inhibition of CCLE and HDG

Compound	Microorganism	Zone of inhibition			Average Value
		Reading 1	Reading 2	Reading 3	
CCLE	<i>E. coli</i>	12	12	12	12
	<i>S. aureus</i>	14	14	11	13
HDG	<i>E. coli</i>	24	24	24	24
	<i>S. aureus</i>	19	18	20	19
Streptomycin	<i>E. coli</i>	24	24	24	24
	<i>S. aureus</i>	25	25	25	25
Tween 80	<i>E. coli</i>	-	-	-	-
	<i>S. aureus</i>	-	-	-	-

Preliminary Phytochemical screening of CCLE

The CCLE was subjected to qualitative testing as per the procedure given in standard references [18,20]. The group of compounds identified in CCLE are given in table 2.

Table 2: Phytoconstituents of the CCLE

S. No.	Tests	Phytoconstituents
1	Alkaloids	+
2	Flavonoids	+
3	Glycosides	+
4	Proteins	-
5	Tannins and Phenolic compounds	+
6	Sterols	+

Where, (+) positive represent presence, and (-) negative represent absence

DISCUSSION

The preliminary phytochemical screening of prepared CCLE revealed presence of alkaloids, flavonoids, glycosides, sterols, tannins, and phenolic compounds. The IR, ¹H-NMR, and mass spectral data of HDG was found to be in agreement with its structure. The characteristic ¹H-NMR signal at 4.33 & 9.50 for NH₂ & NH, appearance of IR band at 3250 cm⁻¹ and m/z value at 242 supported the successful synthesis of HDG. These spectral values were also further confirmed based on the literary facts [152]. Evidence reports *S. aureus* and *E. coli*, to trigger peri-implantitis. The growing incidences of microbial resistance towards conventional antibiotics raises the demand for evaluation of antimicrobials [7-9]. Reports suggests use of *C. citratus* in the treatment of various diseases and to possess strong antimicrobial potential. As per the literature available over different parts of *C. citratus* plant and yet much more has to be explored for this plant. Hence, investigators of present study planned to evaluate the in-vitro inhibition potential of *C. citratus* leaves extract against PCP (*S. aureus* and *E. coli*) using well diffusion method. The CCLE was prepared using hydroalcoholic extract 50%. The prepared CCLE was investigated for anti-microbial activity (using well diffusion method) and phytochemical screening. The CCLE showed good inhibitory effect overgrowth of *S. aureus* and *E. coli*. On the other hand, the HDG was prepared by amination of ester derivative of eugenol, and when tested against PCP (*S. aureus* and *E. coli*) exhibited high inhibitory potential study revealed that synthetic derivative (HDG) possesses high potential when compared with CCLE. The results of the present study were also supported my many other studies [155-182]. However, further preclinical, and clinical studies are required to further support the antimicrobial potential of HDG.

CONCLUSION

The results of the present study over inhibitory potential of HDG and CCLE against PCP, it is here by concluded that synthetic derivative HDG possess high antimicrobial potential against PCP especially *S. aureus* and *E. coli*. Present study recommends that highly potent HDG should be further evaluated based on the preclinical and clinical data.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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